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Death of Enterohemorrhagic *Escherichia coli* O157:H7 in Real Mayonnaise and Reduced-Calorie Mayonnaise Dressing as Influenced by Initial Population and Storage Temperature

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This study was undertaken to determine the survivability of low-density populations (10^0 and 10^2 CFU/g) of enterohemorrhagic *Escherichia coli* O157:H7 inoculated into real mayonnaise and reduced-calorie mayonnaise dressing and stored at 20 and 30°C, temperatures within the range used for normal commercial mayonnaise distribution and storage. Inactivation patterns at 5°C and inactivation of high-inoculum populations (10^6 CFU/g) were also determined. The pathogen did not grow in either mayonnaise formulation, regardless of the inoculum level or storage temperature. Increases in storage temperature from 5 to 20°C and from 20 to 30°C resulted in dramatic increases in the rate of inactivation. Populations of *E. coli* O157:H7 in the reduced-calorie and real formulations inoculated with a population of 0.23 to 0.29 $\log_{10}$ CFU/g and held at 30°C were reduced to undetectable levels within 1 and 2 days, respectively; viable cells were not detected after 1 day at 20°C. In mayonnaise containing an initial population of 2.23 $\log_{10}$ CFU/g, viable cells were not detected after 4 days at 30°C or 7 days at 20°C; tolerance was greater in real mayonnaise than in reduced-calorie mayonnaise dressing stored at 5°C. The tolerance of *E. coli* O157:H7 inoculated at the highest population density (6.23 $\log_{10}$ CFU/g) was less in reduced-calorie mayonnaise dressing than in real mayonnaise at all storage temperatures. In reduced-calorie mayonnaise dressing and real mayonnaise initially containing 2.23 $\log_{10}$ CFU/g, levels were undetectable after 28 and 58 days at 5°C, respectively. When *E. coli* O157:H7 was inoculated at a population of 6.23 $\log_{10}$ CFU/g, it was not detected in reduced calorie-mayonnaise dressing held at 5°C after 58 days and was approaching undetectable levels in real mayonnaise after 93 days. The pathogen clearly does not survive in real mayonnaise or reduced-calorie mayonnaise dressing commercially prepared with good manufacturing practices, and the rate of inactivation is most rapid at temperatures at which commercially processed mayonnaise is distributed and stored.

Enterohemorrhagic *Escherichia coli* O157:H7 was first identified as a food-borne pathogen in 1982 and has since been documented as causing illness on an international scale (5). The pathogen is known to cause hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (14).

Undercooked ground beef has been the principal vehicle for *E. coli* O157:H7 (5, 9, 14). However, acidic foods have also been implicated in outbreaks of *E. coli* O157:H7 infection. In 1991, unpasteurized apple cider was linked to an outbreak of diarrhea and hemolytic uremic syndrome in Massachusetts (3). Studies by Zhao et al. (23) revealed that *E. coli* O157:H7 inoculated into apple cider at pH 3.6 to 4.0 can survive for as long as 31 days at 8°C. Miller and Kaspar (11) reported that *E. coli* O157:H7 inoculated into apple cider (pH 3.7 to 4.1) was detectable after 14 to 21 days at 4°C. One strain survived at pH 2 in Trypticase soy broth, with a minimal decrease in the viable-cell population after 24 h at 4°C.

In March 1993, 40 to 50 people became ill with *E. coli* O157:H7 infection after eating in restaurants of the same chain in Oregon. The illness was epidemiologically linked to salad dressing made in these restaurants with commercial mayonnaise (10). Under federal regulations, commercially manufactured mayonnaise made with unpasteurized eggs must have a pH of less than or equal to 4.1, have an acetic acid concentration in the aqueous phase of greater than or equal to 1.4%, and be held for a period of 72 h before being released for use as a food product (19). On the basis of studies by Wethington and Fabian (21) indicating that acetic acid was the ingredient in mayonnaise and salad dressing with the greatest influence on killing salmonellae, these regulations were established to ensure the destruction of salmonellae. In the early 1970s, most manufacturers of commercial mayonnaise stopped using unpasteurized eggs in mayonnaise and began using pasteurized eggs certified by the U.S. Department of Agriculture. More recently, to satisfy consumer demand for less-tart, reduced-calorie mayonnaise dressing, formulations have been modified to result in 0.7% acetic acid in the aqueous phase. Erickson and Jenkins (6) and Glass and Doyle (7) investigated the behavior of salmonellae and *Listeria monocytogenes* in various commercial mayonnaise products and concluded that properly acidified (pH 4.1) reduced-calorie mayonnaise containing 0.7% acetic acid in the aqueous phase is microbiologically safe. Acetic acid is more bactericidal than citric acid to salmonellae, *Clostridium perfringens*, and *Staphylococcus aureus* in homemade mayonnaise (16).

The 1993 outbreak of *E. coli* O157:H7 infection linked to store-made salad dressing, coupled with the pathogen's extraordinary resistance to acidic pHs, has raised questions about its ability to survive in various mayonnaise formulations. Studies in our laboratory (22) revealed that *E. coli* O157:H7 inoculated into mayonnaise at a population of 3.82 $\log_{10}$ CFU/g survived for 34 to 55 days at 5°C, depending upon the lot. Similar results were obtained with a higher inoculum (6.82

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$\log_{10}$ CFU/g). Weagant et al. (20) studied survival patterns of *E. coli* O157:H7 inoculated into mayonnaise and mayonnaise-based sauces at populations of 7.16 to 7.98 $\log_{10}$ CFU/g. The pathogen was detectable for up to 35 days in mayonnaise stored at 7°C and past 35 days in three of four sauces held at 5°C. Raghubeer et al. (17) inoculated mayonnaise with *E. coli* O157:H7 cells at a population of $>10^6$ CFU/g. The pathogen was not detected after the mayonnaise was stored at 22°C for 96 h. None of these studies examined the survival of low-density populations of *E. coli* O157:H7 in mayonnaise at non-refrigeration temperatures.

The objective of this study was to determine the survivability of low (10^0 and 10^2 CFU/g) as well as high (10^6 CFU/g) populations of *E. coli* O157:H7 inoculated into real mayonnaise and reduced-calorie mayonnaise dressing as affected by temperatures (20 and 30°C) within the range of those at which commercial mayonnaise would be kept during storage, distribution, and marketing. Survivability at 5°C was also studied to replicate the parameters used in previous studies.

MATERIALS AND METHODS

Mayonnaise. Commercially manufactured real mayonnaise and reduced-calorie mayonnaise dressing were used. Subsamples (2,000 g) were deposited in 3.77-liter (1-gallon) Zip-Loc freezer bags. Samples were also analyzed for pH and populations of aerobic mesophilic microorganisms, yeasts and molds, lactic acid bacteria, and *E. coli* O157:H7. The titratable acidity of each sample was determined by the procedure described by Troller and Scott (18).

***E. coli* O157:H7 strains and preparation of inoculum.** A six-strain mixture of *E. coli* O157:H7 (strains C7927 [human isolate], E09 [meat isolate], E0019 [calf fecal isolate], F500 [human isolate], 932 [human isolate], and 933 [beef isolate]) was used as an inoculum. Each strain was individually cultured at 37°C in 10 ml of tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.). Three consecutive 24-h loop transfers were made in 100 ml of TSB, which served as suspensions to prepare the inoculum. Cells were sedimented by centrifugation (4,000 × *g*, 15 min), resuspended, and washed three times with 0.05 M phosphate buffer (pH 6.9). The last sediment was suspended in 100 ml of 0.04 M phosphate buffer containing 0.75% sodium chloride (PBS). Samples of the suspensions of each strain (5 ml each) were combined to yield a cocktail containing approximately equal populations of each strain.

Inoculation of mayonnaise. Twenty milliliters of diluted (PBS) inocula containing ca. 10^8, 10^4, or 10^2 CFU of *E. coli* O157:H7 cells per ml was added to 2,000 g of mayonnaise to give populations of ca. 10^6, 10^2, and 10^0 CFU/g, respectively. Control samples were prepared by adding 20 ml of sterile PBS to 2,000 g of mayonnaise. Inoculated and control mayonnaise preparations were thoroughly mixed by hand for 3 min to ensure a homogeneous distribution of *E. coli* O157:H7 and PBS. Subsamples (140 to 150 g) of inoculated mayonnaise were aseptically transferred to sterile 8-oz. (ca. 240-ml) glass jars and placed in incubators at 5, 20, or 30°C.

Procedures for analysis. Populations of each strain of *E. coli* O157:H7 in washed suspensions and in the diluted (10^0, 10^{-1}, 10^{-5}, and 10^{-7}) cocktail were determined by serial (1:10) dilution in sterile 0.1% peptone and surface plating (0.1 ml) in duplicate on sorbitol MacConkey agar no. 3 (SMA) (Unipath Co., Oxoid Division, Ogdensberg, N.Y.) supplemented with 0.1% 4-methylumbelliferyl-β-D-glucuronide and on tryptic soy agar (TSA) (Difco). Colonies were counted after the plates were incubated for 16 to 22 h at 37°C.

At the initiation of experiments (day 0) and after selected times of storage at 5, 20, or 30°C, triplicate 5-g portions of mayonnaise were combined with 10 ml of modified TSB (mTSB) (14) in sterile 50-ml conical centrifuge tubes and thoroughly mixed. Samples (1.0 ml) were removed for surface plating on SMA as described above (duplicate 0.1-ml samples or quadruplicate 0.25-ml samples [10^0 dilution]) for enumeration of *E. coli* O157:H7. When samples required enrichment, the homogenized mixture of mayonnaise and mTSB was combined with 90 ml of mTSB, vigorously shaken, and incubated at 37°C for 24 h. Serially (1:10) diluted samples (0.1 ml) were surface plated in duplicate on SMA. The plates were incubated at 37°C for 16 to 22 h before colonies were counted. Colonies (five per plate) were randomly selected for confirmation by the *E. coli* O157:H7 latex agglutination test (Unipath) and the API 20E diagnostic test (bioMerieux Vitek, Hazelwood, Mo.).

This analysis scheme resulted in three lower limits of detection of *E. coli* O157:H7, depending on the procedure employed. The lower limit of detection in 0.1 ml of a homogenate containing 5.0 g of mayonnaise and 10 ml of mTSB plated on SMA was one viable cell per 0.066 g (duplicate 0.033-g samples) of mayonnaise, the lower limit when a total of four 0.25-ml portions of homogenate were plated on SMA was one viable cell in 0.33 g of mayonnaise, and the lower limit after enrichment in mTSB was one viable cell in 5 g of mayonnaise. Results are reported on this basis.

TABLE 1. Formulas for real mayonnaise and reduced-calorie mayonnaise dressing[a]

Ingredient	% (wt/wt) in:	
	Real mayonnaise	Reduced-calorie mayonnaise dressing
Soybean oil	78.99	31.20
Water	7.10	51.09
Starch		4.50
Egg yolk, 10% salt	6.00	
Whole eggs, 10% salt, yolk fortified		7.50
Sugar	4.00	0.60
Vinegar, 120 grain	3.00	3.42
Salt	0.60	1.10
Mustard flour	0.30	0.30
Xanthan gum		0.20
Sodium benzoate		0.09
EDTA	0.01	
Total	100.00	100.00

[a] The formulas were obtained from the Association of Dressings and Sauces.

At days 0, 9, 31, and 93, mayonnaise was assayed for populations of aerobic mesophilic microorganisms (plate count agar; 30°C, 48 h), lactic acid bacteria (APT agar [Difco] acidified with sterile 10% tartaric acid to pH 4.0; 37°C, 3 days), and yeasts and molds (dichloran rose bengal chloramphenicol agar [DRBC] [Unipath]; 25°C, 5 days). In all cases, samples (5 g) serially (1:10) diluted in sterile 0.1% peptone were surface plated in duplicate (0.1 ml) or quadruplicate (0.25 ml) on appropriate enumeration media.

Statistical analysis. Mean values from duplicate samples from three replicates are reported, with the exception of analyses in which quadruplicate 0.25-ml samples of homogenates of mayonnaise and primary diluent were plated on enumeration media. In the latter case, the sum of values from each set of quadruplicate samples represents one replicate value. Mean values, presented as CFU per gram of mayonnaise were compared for significant differences ($P < 0.05$) by using analysis of variance and Duncan's multiple-range test (SAS Institute, Cary, N.C.).

RESULTS

The compositions of real mayonnaise and reduced-calorie mayonnaise dressing are listed in Table 1. The pH and titratable acidity values for the real mayonnaise used in various replicate experiments ranged from 3.86 to 3.97 and 0.43 to 0.44%, respectively. The pH of the reduced-calorie mayonnaise dressing was 4.08, and its titratable acidity was 0.46%. Neither the pHs nor the titratable acidities of the two formulas were affected by adding inocula. Changes in pH during storage ranged from −0.07 to +0.01 U during storage for up to 93 days. The titratable acidity was essentially unchanged during the same storage period.

Populations of the six test strains of *E. coli* O157:H7 in unwashed and washed 24-h cultures and in the six-strain mixtures of suspensions of washed cells used in experiments are shown in Table 2. The range in values reflects differences in populations in the inocula used in all experiments.

No *E. coli* O157:H7, aerobic mesophiles, lactic acid bacteria, or yeasts and molds were detected in uninoculated mayonnaise at the initiation of any of the experiments. The populations of aerobic mesophiles in inoculated samples (Table 3) generally reflected those of *E. coli* O157:H7. Except for an occasional mold colony appearing on DRBC agar, probably resulting from airborne contaminants, no yeasts or molds and no lactic acid bacteria were detected in uninoculated or inoculated mayonnaise throughout the incubation.

The inactivation patterns of *E. coli* O157:H7 as affected by the initial populations in inocula, type of mayonnaise, and storage temperature are shown in Table 4. Populations of *E.*

TABLE 2. Populations of *E. coli* O157:H7 in 24-h cultures and after washing in 0.1 M phosphate buffer

Strain(s)	Population (log_{10} CFU/ml)			
	Before washing		After washing	
	TSA[a]	SMA[b]	TSA	SMA
C7927	8.88	8.93–9.07	9.36	9.24–9.31
E09	9.36	9.33–9.32	9.43	9.09–9.42
E0019	9.26	9.11–9.15	9.25	8.97–9.18
F500	9.04	9.01–9.46	9.12	9.15–9.53
932	8.97	8.88–9.27	8.95	8.95–9.41
933	9.31	9.13–8.77	9.33	9.00–9.29
Mixture of all six strains			9.18	9.23–9.29

[a] TSA, tryptic soy agar.
[b] The range reflects differences in populations in inocula used in all experiments.

coli O157:H7 in reduced-calorie mayonnaise dressing and real mayonnaise inoculated at a population of 0.23 to 0.29 log_{10} CFU/g and held at 30°C were reduced to undetectable levels within 1 and 2 days, respectively; viable cells were not detected after 1 day at 20°C. In both formulas containing an initial inoculum of 2.23 log_{10} CFU/g, viable cells were not detected after 4 days at 30°C or at 7 days at 20°C; tolerance was higher in real mayonnaise than in reduced-calorie mayonnaise dressing stored at 5°C. At the highest inoculum level (6.23 log_{10} CFU/g), viable *E. coli* O157:H7 cells were not detected in reduced-calorie mayonnaise dressing or real mayonnaise after 4 or 7 days of incubation at 30°C, respectively.

When *E. coli* O157:H7 was inoculated at populations of 2.23 or 6.23 CFU/g, its survival was longer when mayonnaise was incubated at 20°C than when mayonnaise was incubated at 30°C. The most marked increase in survival time occurred in real mayonnaise containing the highest initial inoculum (6.23 log_{10} CFU/g). The rate of inactivation of *E. coli* O157:H7 was further decreased by storing inoculated mayonnaise at 5°C, a temperature much lower than that used to manufacture, distribute, and store commercial mayonnaise. In reduced-calorie mayonnaise dressing and real mayonnaise initially containing 2.23 log_{10} CFU/g, viable cells were not detected after 28 and 58 days of storage at 5°C, respectively. When inoculated at a population of 6.23 log_{10} CFU/g, *E. coli* O157:H7 was not detected in reduced-calorie mayonnaise dressing held at 5°C for 58 days and was approaching undetectable levels in real mayonnaise after 93 days.

TABLE 3. Populations of aerobic mesophilic microorganisms in real mayonnaise and reduced-calorie mayonnaise dressing inoculated with *E. coli* O157:H7 and stored for 0, 9, 31, or 93 days at 5, 20, or 30°C[a]

Inoculum (log_{10} CFU/g)	Storage temp (°C)	Type of mayonnaise	Population (log_{10} CFU/g) on day:			
			0	9	31	93
0.23–0.29	5	Real	0.30	ND[b]		
		Reduced[c]	1.08	1.00		
	20	Real	0.30	ND		
		Reduced	1.08	1.15		
	30	Real	0.30	ND		
		Reduced	1.08	ND		
2.23	5	Real	1.98	0.30	ND	ND
		Reduced	2.03	1.16	1.18	
	20	Real	1.98	0.85	1.08	
		Reduced	2.03	0.60	1.26	
	30	Real	1.98	ND	0.95	
		Reduced	2.03	0.48	0.70	
6.23	5	Real	5.89	4.95	3.95	1.01
		Reduced	6.20	5.42	4.81	0.87
	20	Real	5.89	4.39	0.78	ND
		Reduced	6.20	2.93	1.20	
	30	Real	5.89	0.70	ND	
		Reduced	6.20	0.90	0.90	

[a] The inoculum contained a mixture of six strains (see Table 2).
[b] ND, not detected in three 0.33-g samples.
[c] Reduced-calorie mayonnaise dressing.

DISCUSSION

The ability of *E. coli* O157:H7 to tolerate an acid pH in several types of foods has been documented by other researchers. *E. coli* O157:H7 can grow at acidities approaching 0.8% (pH < 5) during the manufacture of cottage cheese (2). Sixteen cases of *E. coli* O157:H7 infection have been linked to the consumption of flavored yogurt (12), which contains lactic acid as a result of fermentation.

Nettles-Cutter and Siragusa (13) studied the efficacy of acetic, lactic, and citric acids for controlling *E. coli* O157:H7 attached to beef carcass tissue. The type of acid was not a significant treatment factor. The reduction of the surface pH from 6.57 to as low as 3.55 (3% citric acid) upon application of acids was suggested as a factor contributing to inactivation of *E. coli* O157:H7. Brackett et al. (4), on the other hand, reported that the bactericidal effect of organic acids sprayed onto the surface of beef tissue was minimal. However, the lowest surface pH resulting from spray application was 5.05 (1.5% lactic acid), a value at which the test acids would not be completely undissociated and, therefore, may be less lethal. *E. coli* O157:H7 remained viable in sausage fermented to pH 4.8 and stored at 4°C for 2 months (8) and in beef salad (pH 5.4) containing 40% mayonnaise which was held at 5°C for 3 days (1).

At the highest inoculum level (6.23 log_{10} CFU/g) used in the first set of experiments, *E. coli* O157:H7 was detected in real mayonnaise stored at 20 or 30°C for 17 or 4 days, respectively (Table 4). Death was more rapid in reduced-calorie mayonnaise dressing, with undetectable levels occurring at 11 and 4 days when storage was at 20 and 30°C, respectively. These inactivation rates are not as rapid as the inactivation rate reported by Weagant et al. (20). Those researchers observed that an initial population of 7.16 to 7.23 log_{10} CFU/g of mayonnaise was reduced to an undetectable level after storage for 3 days at 25°C. The pH of the mayonnaise used in that study was lower (3.65) than that of the reduced-calorie mayonnaise dressing (pH 4.04 to 4.08) used in our study, thus possibly accounting for the different rates of death. Survival of *E. coli* O157:H7 in blue-cheese dressing (pH 4.44) and seafood sauce (pH 4.38) stored at 7°C was reported to be greater than that in mayonnaise-mustard sauce (pH 3.68) and Thousand Island dressing (pH 3.76) (20).

In our study, the behavior of *E. coli* O157:H7 in mayonnaise inoculated with the lowest test inoculum (0.23 to 0.29 log_{10} CFU/g) and held at 5°C did not appear to follow the same trend observed with the highest inoculum; i.e., resistance to inactivation of cells in the lowest test inoculum appeared to be greater in reduced-calorie mayonnaise dressing than in real mayonnaise. The lower pH (3.86 to 3.97) of real mayonnaise may have had an immediate lethal effect on a higher number of the most acid-sensitive cells of *E. coli* O157:H7 in the inoculum, in contrast to the case with the higher pH (4.04 to 4.08) of

TABLE 4. Populations or presence of *E. coli* O157:H7 in real mayonnaise and reduced-calorie mayonnaise dressing as influenced by initial inoculum and storage at 5, 20, or 30°C

Initial inoculum (log_{10} CFU/g)	Storage time (days)	Population (log_{10} CFU/g)[a] in:					
		Real mayonnaise			Reduced-calorie mayonnaise dressing		
		5°C	20°C	30°C	5°C	20°C	30°C
0.23–0.29	0[b]	(3)	(3)	(2)	(3)	(3)	(3)
	1	(0)	(0)	(1)	(1)	(0)	(0)
	2	(0)	(0)	(0)	(1)	(0)	(0)
	3	(0)	(0)	(0)	(0)	(0)	(0)
	4	(0)	(0)	(0)	(0)	(0)	(0)
	7	(0)	(0)	(0)	(0)	(0)	(0)
	9	(0)	(0)	(0)	(0)	(0)	(0)
	11	(0)	(0)	(0)	(0)	(0)	(0)
	14						
	17						
	21						
	24						
2.23	0	a 1.60 (3)	1.60 (3)	1.60 (3)	a 1.95 (3)	a 1.95 (3)	1.95 (3)
	1	a 1.00 (3)	ND[c] (3)	ND[c] (3)	a 2.32 (3)	ab 1.69 (3)	ND[c] (3)
	2	a 1.48 (3)	1.00 (3)	ND[c] (3)	ab 1.74 (3)	a 1.74 (3)	ND[c] (0)
	3	a 1.30 (3)	0.70 (2)	ND[c] (2)	b 1.54 (3)	bc 1.30 (2)	ND[c] (1)
	4	a 1.00 (3)	ND[c] (3)	ND[c] (0)	ab 1.74 (3)	c 0.69 (3)	ND[c] (0)
	7	(3)	(0)	(0)	(3)	(0)	(0)
	9	(3)	(0)	(0)	(3)	(0)	(0)
	11	(3)	(0)	(0)	(3)	(0)	(0)
	14	(2)	(0)		(3)	(0)	
	17	(3)	(0)		(3)	(0)	
	21	(2)			(3)		
	24	(2)			(1)		
	28	(1)			(0)		
	31	(0)			(0)		
	37	(1)			(0)		
	44	(0)					
	51	(1)					
	58	(0)					
	65	(0)					
	79	(0)					
	93	(0)					
6.23	0	a 6.26	a 6.26	a 6.26	a 5.94	a 5.94	a 5.94
	1	b 5.37	b 5.18	b 4.56	b 5.67	ab 5.67	b 4.29
	2	c 5.29	c 4.95	c 4.06	b 5.67	bc 5.35	c 1.00
	3	c 5.10	cd 4.79	d 3.32	b 5.56	cd 5.11	c 0.70
	4	c 5.00	d 4.59	e 0.48 (3)	bc 5.47	d 4.78	ND[d] (0)
	7	d 4.67	e 4.06	ND[d] (0)	cd 5.32	e 3.92	ND[d] (0)
	9	d 4.60	f 3.91	ND[d] (0)	de 5.13	f 1.40	ND[d] (0)
	11	e 4.30	f 3.96	ND[d] (0)	e 5.11	ND[c] (0)	ND[d] (0)
	14	g 3.33	g 1.96 (3)		ef 4.98	ND[d] (0)	
	17	f 3.97 (3)	h 0.48 (3)		fg 4.80	ND[d] (0)	
	21	f 3.93 (3)	(0)		g 4.65	(0)	
	24	f 3.89 (3)	(0)		h 4.51		
	28	g 3.57 (3)	(0)		i 3.89		
	31	g 3.72 (3)	(0)		i 3.93		
	37	h 3.25 (3)			j 3.12 (0)		
	44	i 2.95 (3)			k 1.73 (0)		
	51	j 2.44 (3)			l 0.47 (0)		
	58	j 2.28 (3)			ND[d] (0)		
	65	k 1.85 (3)			ND[d] (0)		
	79	l 0.95 (3)			ND[d] (0)		
	93	ND[d] (1)			(0)		

[a] Values in the same column within the same inoculum level that are not preceded by the same letter are significantly different ($P < 0.05$). Numbers in parentheses indicate the number of 5-g samples, of three replicates analyzed, that contained viable *E. coli* O157:H7 as determined by enrichment in mTSB.

[b] Day 0 samples were analyzed 20 to 40 min after inoculation.

[c] Not detected in six 0.033-g samples.

[d] Not detected in three 0.33-g samples.

reduced-calorie mayonnaise dressing. In real mayonnaise receiving the lowest inoculum (0.23 to 0.29 $\log_{10}$ CFU/g) and stored at 5°C, this may have resulted in our inability to detect the organism after 1 day of storage; viable cells were detected in reduced-calorie mayonnaise dressing stored at 5°C for 2 days.

Since the titratable acidity of the real mayonnaise was lower than that of the reduced-calorie mayonnaise dressing, one might expect that the pH of the real mayonnaise would be higher than that of the reduced-calorie product. Titratable acidity is calculated on a weight/weight (acid/mayonnaise) ratio basis. Acetic and other acids partition into the nonfat phase of mayonnaise, mostly into the water phase. Since reduced-calorie mayonnaise dressing contains about 7.2 times more water than real mayonnaise but only 1.1 times more vinegar, the percentage of acetic acid (weight/weight ratio basis) in the aqueous phase of reduced-calorie mayonnaise dressing is substantially less than the percentage in the aqueous phase of real mayonnaise. Thus, even though the titratable acidity of reduced-calorie mayonnaise is higher than that of the real mayonnaise on a weight/weight ratio basis, the titratable acidity in the aqueous phase of reduced-calorie mayonnaise would be considerably lower. Hence, the pH of the reduced-calorie mayonnaise dressing is higher than the pH of real mayonnaise because the H^+ concentration is much lower in the aqueous phase of the reduced-calorie mayonnaise dressing than in that of real mayonnaise as a result of dilution. If a certain percentage of *E. coli* O157:H7 cells are sensitive to low pH and high acidity, these cells would be expected to die soon after inoculation into mayonnaise. The death of this population would be more rapid in real mayonnaise than in reduced-calorie mayonnaise dressing, thus resulting in the apparent elimination of viable *E. coli* O157:H7 cells more quickly in real mayonnaise. This phenomenon was evident in real mayonnaise and reduced-calorie mayonnaise dressing inoculated with 0.23 to 0.29 CFU/g and stored at 5°C but not 20 or 30°C. This observation has no practical implication, however, since commercial mayonnaise is held at nonrefrigeration temperatures during distribution and marketing. The observations on the behavior of high populations of *E. coli* O157:H7 in real mayonnaise and reduced-calorie mayonnaise dressing reported here confirm the results of a previous study in our laboratory (22). In neither study did the pathogen grow in mayonnaise, regardless of the initial inoculum population. Others (17, 20) have likewise demonstrated that *E. coli* O157:H7 inoculated into mayonnaise at populations of $>10^6$ CFU/g dies rapidly when held at 22 to 25°C. Our studies reveal that increases in storage temperature from 5 to 20°C and from 20 to 30°C resulted in a dramatic acceleration in the rate of inactivation.

The tolerance of *E. coli* O157:H7 inoculated at the two highest test populations was greater in real mayonnaise than in reduced-calorie mayonnaise dressing held at 20 or 30°C, temperatures within the range used for normal commercial mayonnaise distribution and storage, despite the lower pH and actual higher acidity in the nonfat phase of real mayonnaise than in that of reduced-calorie mayonnaise dressing. This indicates that factors other than pH and acids may be influencing the viability of cells in the two formulas. The presence of 0.09% sodium benzoate in reduced-calorie mayonnaise dressing would be expected to adversely affect the viability of *E. coli* O157:H7. Studies of apple cider (pH 3.6 to 4.0) have revealed that the addition of 0.1% sodium benzoate increased the rate of inactivation of *E. coli* O157:H7 (23). The higher fat content in real mayonnaise may have protected cells against inactivation. Any or all of these factors may be partly responsible for the more rapid inactivation of the pathogen in reduced-calorie mayonnaise dressing than in real mayonnaise receiving the highest level of test inocula.

Results from these and other studies (17, 20, 22) clearly demonstrate that *E. coli* O157:H7 will not survive in real mayonnaise or reduced-calorie mayonnaise dressing commercially prepared with good manufacturing practices. Regardless of the inoculum level, death is most rapid at temperatures (20 to 30°C) at which mayonnaise is stored, distributed, and offered for sale in the marketplace. However, if either type of mayonnaise is cross-contaminated by foods such as raw beef, unclean utensils, or *E. coli* O157:H7-infected food handlers after commercially processed containers are opened, the pathogen may survive at 5°C for several weeks. The responsibility for maintaining commercially manufactured mayonnaises, as well as those prepared in retail or food service facilities, free of *E. coli* O157:H7 is that of the food handler. Good sanitation practices will greatly minimize the possibility of cross-contaminating mayonnaise with *E. coli* O157:H7 and the consequent risk of human infection.

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